

SAMPLE COLLECTION, 7.1.2 PRESERVATION, AND STORAGE

Because sterile conditions must be maintained during collection, preservation, storage, and analysis of indicator bacteria samples, specific procedures have been developed that must be strictly followed. These procedures vary with types of sampling equipment and source of sample (surface water, ground water, treated water, or waste water).

A summary of requirements for sample-collection containers and procedures for sample preservation are given in table 7.1–4.

Adhere strictly to the 6-hour maximum holding-time requirement.

Table 7.1–4. Summary of equipment for sample collection and procedures for sample preservation of indicator bacteria

[EWI, equal-width increment; EDI, equal-discharge increment; L, liter; mL, milliliter; °C, degrees Celsius; EDTA, ethylenediaminetetraacetic acid]

Equipment for sample collection
For EWI or EDI surface-water samples: sterile DH-77 3-L or DH-81 1-L wide-mouth bottle with sterile caps and nozzles.
For surface-water and ground-water samples using point samplers: a sterile, narrow-mouth container, 250- to 500-mL capacity.
All containers must be composed of sterilizable materials such as borosilicate glass, polypropylene, stainless steel, or Teflon™.
Procedures for sample preservation
Chill all samples at 1–4°C before analysis. If necessary, add 0.1 mL of a 10-percent sodium thiosulfate solution per 100 mL sample for halogen neutralization.
Add 0.3 mL of a 15-percent EDTA solution per 100 mL sample for chelation of trace elements, if necessary.
Do not exceed the 6-hour maximum holding time after sample collection.

7.1.2.A SURFACE-WATER SAMPLE COLLECTION

The areal and temporal distribution of indicator bacteria in surface water can be as variable as the distribution of suspended sediment because bacteria commonly are associated with solid particles. To obtain representative data, use the same methods for collecting surface-water samples for bacteria analysis as for suspended sediment (Edwards and Glysson, 1988; NFM 4).

- ▶ For flowing water, use depth-and-width-integrating sampling methods.
- ▶ For still water or other surface-water conditions for which depth-and-width-integrating methods are not applicable, use the hand-dip method.

Quality control. Depending on the data-quality requirements, quality-control (QC) samples (blanks and replicates) can comprise from 5 to 30 percent or more of the total number of samples collected over a given period of time.

- ▶ Collect and analyze field blanks to document that sampling equipment has not been contaminated.
- ▶ Collect and analyze filter and procedure blanks to document that filtration equipment was sterile and not subsequently contaminated by the analyst. The filter blank is processed through the filtration equipment before the sample is filtered and the procedure blank is processed through the filtration equipment afterwards.

Process field blanks before collecting the water sample:

1. Rinse sterile sampling equipment and containers with sterile buffered water.
2. Process sterile buffered water through sampling equipment and into sterile sample bottle and analyze for colony growth. If no growth is observed, the sample was collected using sterile procedures.

Depth-and-width-integrating methods

Depth-and-width-integrating sampling methods include the equal-discharge increment (EDI) method and the equal-width increment (EWI) method. EDI or EWI methods are recommended unless study objectives or site characteristics dictate otherwise.

1. Select the EDI or EWI method. The EDI method is preferred to the EWI method for sites at which the velocity distribution across a stream section is well established; for example, at a gaging station (Edwards and Glysson, 1988).

2. Select the appropriate sampler and equipment. **Sampling equipment must be sterile**—polypropylene collection bottle, nozzle, and cap, or bags for the bag sampler.

- For streams with depths of 5 m or less, use a US D-77 or a US DH-81 sampler.
- For stream sections where depths exceed 5 m, use the bag version of the US D-77, either with autoclavable Teflon™ bags or autoclavable cooking bags. Thermotolerant polymers are described in more detail in 7.1.1 under "Equipment and Equipment Sterilization Procedures".
- For compositing subsamples, use a sterile 3-L bottle with a US D-77 sampler and a sterile 1-L bottle with a US DH-81 sampler.
- Use the proper nozzle size and transit rate for the velocity conditions in the section to ensure isokinetic collection of the sample.

The number of subsamples that can be collected by use of EDI and EWI collection methods are somewhat limited because churn and cone splitters cannot be autoclaved or easily kept sterile under field conditions.

- ▶ For wide channels, several samples, each composed of subsamples composited into 3-L bottles, may be needed.
- ▶ For narrow channels, collect subsamples at 5 to 10 or more vertical locations in the cross section without overfilling the bottle.

Hand-dip method

If the stream depth and (or) velocity is not sufficient to use a depth-and-width integrating method, collect a sample by a hand-dip method. Sampling still water or sampling at depth in lakes, reservoirs, estuaries, and oceans requires a sterile point sampler. Niskin, ZoBell, and Wheaton samplers hold a sterilizable bottle or bag. To collect a hand-dipped sample:

1. Open a sterile, narrow-mouth borosilicate glass or plastic bottle; grasp the bottle near the base, with hand and arm on downstream side of bottle.
2. Without rinsing, plunge the bottle opening downward, below the water surface. Allow the bottle to fill with the opening pointed slightly upward into the current.
3. Remove the bottle with the opening pointed upward from the water and tightly cap it, allowing about 2.5 to 5 cm of headspace (American Public Health Association and others, 1992, p. 9–19; Bordner and Winter, 1978, p. 8). This procedure minimizes collection of surface film and avoids contact with the streambed.

7.1.2.B GROUND-WATER SAMPLE COLLECTION

As with surface water, most bacteria in ground and well water are associated with solid particles. Stable values of field measurements (turbidity, temperature, dissolved-oxygen concentration, pH, and specific electrical conductance) are important criteria for judging if a well has been sufficiently purged for collection of a representative ground-water sample for indicator bacteria analysis (NFM 6.0.2).

Quality control. Depending on the data-quality requirements, quality-control (QC) samples (blanks and replicates) can comprise from 5 to 30 percent or more of the total number of samples collected over a given period of time.

- ▶ Collect and analyze field blanks to document that sampling equipment has not been contaminated.
- ▶ Collect and analyze filter and procedure blanks to document that filtration equipment was sterile and not subsequently contaminated by the analyst. The filter blank is processed through the filtration equipment before the sample is filtered and the procedure blank is processed through the filtration equipment afterwards.

Process field blanks before collecting the water sample:

1. Rinse sterile sampling equipment and containers with sterile buffered water.
2. Process sterile buffered water through sampling equipment and into sterile sample bottle and analyze for colony growth. If no growth is observed, the sample was collected using sterile procedures.

Production and domestic water-supply wells

- ▶ If samples are to be collected from a production well, select a tap that supplies water from a service pipe connected directly to the main and is not served from a cistern or storage tank (American Public Health Association and others, 1992, p. 9–18, 19; Britton and Greeson, 1989, p. 5; Bordner and Winter, 1978, p. 5–16).
- ▶ Before collecting the sample, remove screens, filters, or other devices from the tap.

- ▶ Do not sample from leaking taps.
- ▶ Avoid sampling after downhole chlorination, if possible. Dechlorination is required if you cannot avoid collecting the sample before it has passed through the treatment unit.

Collect a sample directly from the tap into a sterile bottle without splashing or allowing sample bottle to touch the tap.

- ▶ Supply wells ordinarily are equipped with permanently installed pumps. If the well is pumped daily, collect the sample directly from the tap into the sterile container (table 7.1–4) after running tap water to waste for a minimum of 5 minutes and after recording stable field measurements.
- ▶ If the well is infrequently used, run the tap to waste until a minimum of five sets of field measurements are obtained.

Observation and monitoring wells

If a well does not have an in-place pump, obtain samples by use of a portable sampler, such as a submersible pump or a bailer (U.S. Environmental Protection Agency, 1982). Samplers and sample line must be autoclaved, sterilized, or disinfected, followed by flushing with ground-water sample, before sample is collected into sterile bottles.

- ▶ Use autoclavable samplers, if possible. After flushing the sterilized pump lines with sample, collect the sample directly into the sterile sample bottles.
- ▶ Use of disinfectants can interfere with chemical analysis—check data-collection objectives before using a disinfectant.
- ▶ **Disinfectants are corrosive, can damage the metal parts of a pump, and render the pump inadequate for sampling trace elements and other constituents.**

To disinfect a pump:

1. Submerge the pump and pump tubing in a 200-mg/L solution of household laundry bleach. Most bleach is about 5 percent chlorine, but bleach in a container left open for more than 60 days may not be full strength.
2. Circulate the disinfectant through the pump and tubing for 30 minutes.

3. Rinse thoroughly by lowering the pump carefully in the well and pumping well water to waste until chlorine is removed (U.S. Environmental Protection Agency, 1982). **The pump must have a backflow check valve (an antibacksiphon device) to prevent residual disinfectant from flowing back into the well.**

The type of well, its use, construction, and condition, could alter samples for fecal-indicator bacteria analysis.

If the pump cannot be disinfected:

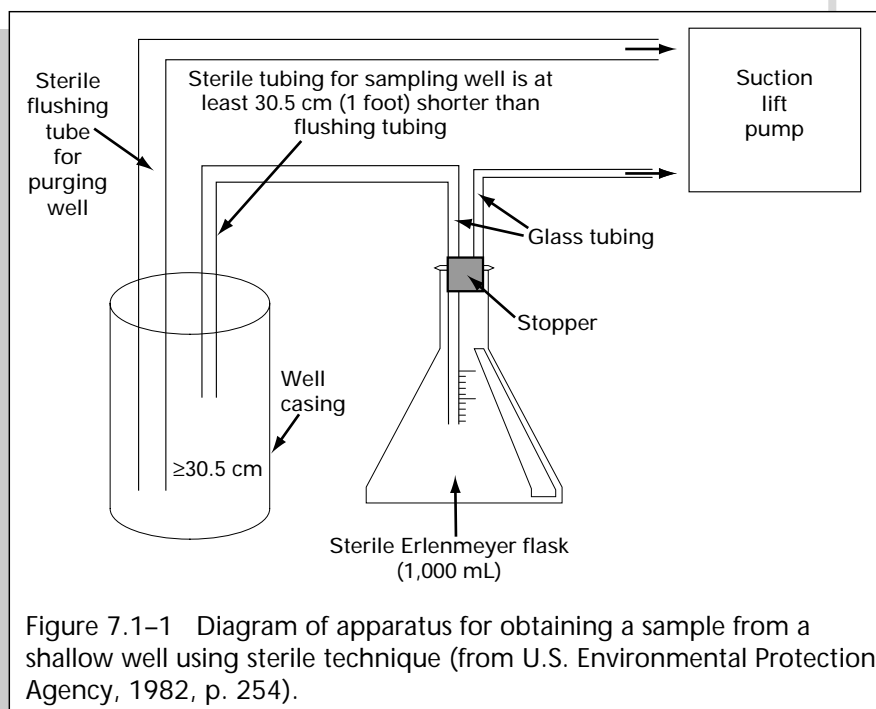
1. Handle the pump and tubing carefully to avoid contamination.
2. Purge the well with the pump used for sampling to allow the pump and tubing to be thoroughly flushed with aquifer water before sampling.
3. Collect blanks through the sampling equipment for quality control at each site.
4. An alternative to sampling with the pump is to withdraw the pump after purging and collect the sample with a sterile bailer (U.S. Environmental Protection Agency, 1982, p. 252–253). If using this method, evaluate the potential for bias from stirring up particulates during pump removal and bailing that otherwise would not be included in the sample.

Presampling activities, such as purging, must be carried out in such a way as to avoid contaminating the well, the equipment, or the samples. Avoid collecting surface film from the well water in the sample and ensure that the sampler intake is at the depth interval targeted for study. If using a nonpumping sampler, select a point-source sampler, such as a bailer with a double-check valve. **Do not use a bailer unless the bailer can be sterilized.**

Sampling equipment that does not require chlorine disinfection:

If water level is less than 7 to 10 m (roughly 20 to 30 ft) below land surface, a sample can be collected without contamination and without chlorine disinfection by use of a surface vacuum pump, a sterile vacuum flask, and two lengths of sterile silicon tubing (U.S. Environmental Protection Agency, 1982), as follows—

1. Obtain and assemble apparatus as shown on figure 7.1–1. To prevent standing water from entering the sampling tube upon insertion into the well, make the sampling tube at least 1 ft shorter than the flushing tube.
2. Autoclave flask and tubing inside a larger autoclavable container or bag before starting field work.
3. Attach tubing to the inlet side of the pump and lower into the well. **Handle the tubing with sterile gloves.**
4. Turn on the pump to the flushing system as the tubing is put into the well and purge the well (NFM 6.0).
5. After purging, turn on the pump to the sample line and collect the sample into the sterile vacuum flask.
6. Transfer the sample from the sterile flask to a sterile sample bottle and keep the sample chilled before processing.



Precautions for collecting samples for microbiological analysis from monitoring wells:

- ▶ Avoid collecting samples from wells with casings made of galvanized materials; such casings can contain bacteriocidal metals. If samples must be collected from these types of wells, add 0.3 mL of a 15-percent EDTA solution per 100 mL of sample.
- ▶ Purge the well (see NFM 6.0.2) while monitoring field measurements. Measurements of turbidity and dissolved oxygen are especially relevant. For wells in which field measurements do not stabilize, proceed with sampling, and use replicate samples for quality control.

Avoid contamination—the maximum contaminant limit for most domestic and public supply wells is exceeded with four total or one fecal coliform or *E. coli* colony per 100 mL sample (USEPA, 1991b).

7.1.2.C SAMPLE PRESERVATION AND STORAGE

After collection, immediately chill samples in an ice chest or refrigerator at 1 to 4°C. Do not freeze samples. Begin analysis as quickly as possible, preferably within 1 hour but not more than 6 hours after sample collection, to minimize changes in the concentration of indicator bacteria.

Chill and store samples in the dark until analysis.